San Fernando Valley State College

RECOMBINATION IN REGIONS ADJACENT TO DELETIONS IN THE X CHROMOSOME OF DROSOPHILA MELANOGASTER

A thesis submitted in partial satisfaction of the requirements for the degree of Master of Science in Biology

by

Linda Brinkley Moore

August, 1967
The thesis of Linda Brinkley Moore is approved:

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Committee Chairman

San Fernando Valley State College

August, 1967
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# TABLE OF CONTENTS

LIST OF TABLES AND FIGURES ........................................... v

Chapter

I. INTRODUCTION ....................................................... 1

II. REVIEW OF THE LITERATURE ...................................... 5

III. MATERIALS AND METHODS ......................................... 15

IV. RESULTS ............................................................. 19

V. DISCUSSION .......................................................... 27

- Recombination in heterozygous deficiencies
- Recombination in homozygous deficiencies
- Cytological and genetic correlations
- Recombination in the presence of insertional duplications

BIBLIOGRAPHY .......................................................... 43
LIST OF TABLES AND FIGURES

Table

<table>
<thead>
<tr>
<th>Table</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Crossing over in the vicinity of deficiencies.</td>
</tr>
<tr>
<td>2. Crossing over in a region homologous to insertional duplications</td>
</tr>
<tr>
<td>3. Crossing over in a region of nonhomology to insertional duplication</td>
</tr>
</tbody>
</table>

Figure

<table>
<thead>
<tr>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Diagrammatic representation of the left (distal) end of the X chromosome, correlating the cytological, genetic, and recombinational properties of the region between yellow and rugose.</td>
</tr>
</tbody>
</table>
ABSTRACT

RECOMBINATION IN REGIONS ADJACENT TO DELETIONS IN THE X CHROMOSOME OF DROSOPHILA MELANOGASTER

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Recombination frequencies were determined in regions adjacent to X-chromosome deletions, both in the heterozygous and the homozygous condition. In heterozygous condition, two separate deletions lying within a region marked by yellow (0.0) and ruby (7.5) produced a striking reduction as compared with values obtained in experiments with structurally normal chromosomes. One deficiency in the homozygous condition, provided with a duplication to prevent lethality, exhibited significantly more recombination in the marked interval than did its heterozygous counterpart. Results from the experiment with the homozygous deficiency were used to provide an accurate estimate of the genetic extent of the deleted region. The observed value closely corresponded with the expectation derived from a combined genetic-cytological unit of measurement, namely, crossover frequency per salivary chromosome band, calculated from existing genetic and cytological data.
Tests of recombination in homozygous deficient females required the introduction of an insertional translocation to permit the survival of the females. Accordingly, the possible interchromosomal effects of three different insertional translocations on recombination in both deficient and structurally normal chromosomes were tested. No interchromosomal effects were shown to stem from their presence. However, a decrease in recombination was obtained in one homologous region of structurally normal chromosomes in the presence of the longest duplication tested, a finding consistent with the theory of competitive pairing. None of the inserted fragments affected recombination in a nonhomologous region of structurally normal chromosomes.
CHAPTER I
INTRODUCTION

The simultaneous occurrence of two or more chromosome breaks in the same nucleus may produce a variety of structural aberrations. Deficiency, the excision of a segment lying between two breakpoints in one chromosome and the concomitant physical shortening of the chromosome by the deleted amount, is a common radiation-induced abnormality. The resulting chromosomal fragment, although lost from its chromosome of origin, need not be lost from the nucleus as a whole. The presence of a third break in a nonhomologous chromosome in the same nucleus may result in the "capture" of the deleted fragment by the nonhomologous chromosome. The genome of such an individual will remain balanced, even though the karyotype is altered, since the chromosomal material deleted from one chromosome is inserted intact into a nonhomologous chromosome of the same nucleus. Deficiencies and insertional translocations of identical lengths provide unique tools for studying recombination in regions surrounding deleted sections.

The presence of a deficient region in one chromosome of a pair results in an alteration of the structural
homology between the two. Since recombination must be preceded by synapsis of homologues, recombination in the region of the chromosome encompassing a deficiency should be reduced by at least the genetic length of the missing segment, more if the deficiency causes synaptic difficulties in its vicinity. Initial studies by Bridges (1917, 1919) on deficiencies in the X chromosome and in the second chromosome (Bridges, Skoog, & Li, 1936) upheld the expectation of a loss of recombination corresponding to the genetic length of the missing region, but did not demonstrate significant linkage disturbances in areas adjacent to the deletion. This result was also reported by Mohr (1923) in the course of extensive studies on a Notch deficiency in the X chromosome. These investigations would seem to imply that deficiencies short enough to be viable and fertile in the heterozygous female do not cause synaptic difficulties sufficiently great to be detected by their effects on recombination in adjacent areas.

Deficiencies can be analyzed not only genetically, but also cytologically. Observations on salivary chromosome preparations of females heterozygous for a deficiency reveal the characteristic synaptic pattern associated with the deficient chromosome (Bridges, et al., 1936). The normal homologue pairs as closely as possible with its deficient partner, creating a loop with the excess of
chromosomal material in order to do so. Although somatic pairing of polytene chromosomes and meiotic synapsis preceding recombination have not been shown to be identical, the general pairing patterns of homologous elements are probably similar. The contortions that the normal homologue must go through in order to pair with a structurally aberrant mate often can be seen in salivary chromosomes to result in some degree of asynapsis in regions immediately adjacent to the breakpoints of the deficiency. Interference by deficiencies with synapsis in meiotic chromosomes, however, is not borne out by the recombination studies of previous investigators.

This long-standing inconsistency between genetic information obtained by early workers and expectations derived from cytological observations has received little if any attention in extensive later published investigations on the effects of chromosomal aberrations on recombination (Sturtevant and Beadle, 1936; Steinberg and Fraser, 1944; Schultz and Redfield, 1951). Studies on duplications (Dobzhansky, 1934; Grell, 1964) have correlated effects on recombination with the length of the duplicating fragment. Environmental factors known to influence recombination, such as age, temperature, sex (see reviews of Swanson, 1957, 1967) and position of the genes relative to the centromere (Beadle, 1932; Grell, 1964)
have also been studied.

The present study was undertaken to provide quantitative information on recombinational values in the vicinity of deletions. The results of tests with two separate deficiencies, showing reduced recombination in their vicinities, were in accord with expectations from cytological observations of synaptic difficulties. Insertional translocations, acting as duplications, were employed to permit further study of recombination in the vicinity of homozygous deficiencies, free from the possible influence of nonhomology between partner chromosomes on recombination. In these studies, normal recombinational values were found.
CHAPTER II

REVIEW OF THE LITERATURE

The existing literature concerning the effects of deletions on recombination is sparse. Deficiencies were first associated with phenotypic effects, later with the actual loss of chromosomal material. Their prime usefulness as genetic tools resides in the opportunity they provide to localize genes to a particular physical position on the chromosome. Overlapping deficiencies were used by Slizynska (1938) to analyze the white-facet region of the X chromosome. The published studies on deletions largely concern themselves with localizing a missing segment to a particular region of the linkage map. Only coincidentally were the effects of deletions on recombination in surrounding regions reported. Few studies have been undertaken to specifically test the recombinational effects that might occur from the loss of a section of chromosome. The answer seems self-evident: recombination should be reduced by an amount equivalent to the length of genetic material removed.

The earliest report of the deficiency phenomenon was by Bridges (1917). It was primarily concerned with locating the deleted segment by genetic tests. At that
time, salivary chromosome analysis was unknown; consequently, any accurate cytological analysis of the deficient chromosome was impossible. The deleted segment was localized within a short interval (rudimentary, 55.1, to fused, 59.5) of 4.4 crossover units. Since Bridges found a 0.70% reduction in recombination in this interval in heterozygous deficient females, he concluded that this represented the actual genetic extent of the missing chromosomal material. Later, a salivary chromosome analysis of the deficient chromosome by Sutton (Bridges and Brehme, 1944) revealed that the deletion had actually occurred in a chromosome which contained the tandem duplication, Bar. Although the 16Al-7 repeat was removed by the deficiency, this merely served to restore the wild type condition at the Bar "locus." The only material actually deleted, as compared with a normal nonduplicated chromosome, was a 10-band salivary chromosome section, 15Fl-9, inclusive.

A later investigation by Bridges (1919) of a vermilion deficiency failed to localize the deficiency or define its extent. The stock was lost before a salivary chromosome analysis could be made.

An extensive investigation of the Notch-8 (N\textsuperscript{B}) deficiency was carried out by Mohr (1923). No salivary chromosome analysis was possible at the time of this
study. However, Mohr did observe oogonial division figures and reported that "... the central ends of the X chromosomes of \( N^8 \) females are slightly unlike each other" ("central" being defined as the left end of the genetic map). No quantitative estimates of the amount of chromosome material lost were possible from these cytological observations. Later, salivary chromosome analyses by various workers (Mackensen, Gottschewski, and Slizynska, cited in Bridges and Brehme, 1944) showed that 18 bands had been removed from the distal region of the chromosome. The results of genetic tests by Mohr showed that the deleted segment reduced recombination in the surrounding region by only the presumed genetic length of the missing segment. Although not all of his results were used in arriving at this conclusion, Mohr included the complete data obtained in his genetic tests in an appendix to the paper. An examination of the results of some of these tests indicate that the investigator was not aware of the existence of factors that could influence the outcome of his experiments. Long unmarked intervals were measured; some markers were used in combinations which have since been discovered to modify their phenotypic expression.

In a somewhat later paper, Bridges, Skoog, and Li (1936) reported on both cytological and genetic properties
of a rather long 50-band deficiency in the second chromosome. Salivary chromosome drawings of the heterozygous deficiency illustrated synaptic difficulties between the deficient chromosome and its normal homologue. Genetic tests showed recombination to be reduced by 1.5% in the regions surrounding the deficiency. In order to calculate a theoretical expectation for comparison, Bridges, et al., utilized the total micron length of the salivary chromosome in combination with its total genetic length. The resulting measurement of crossover frequency per micron gave an estimated value of 1.2% for the deleted region. This expected reduction of recombination corresponded closely to the 1.5% reduction actually observed in genetic tests. The highly localized variability of crossover frequencies per unit of physical length along the chromosome was not then fully realized. Bridges and co-workers were aware of the limitations of their study, however, and accordingly admitted the possibility that the reduction in recombination observed in deficiency heterozygotes could represent more than the genetic extent of the missing segment.

The results obtained by previous investigators do not permit a definitive statement of the effects of deletions on recombination. The conclusions drawn by the investigators must be viewed as tentative when their
results are examined in the light of knowledge gained during the three decades of genetic research since 1936. The early findings do not, in fact, refute the existence of linkage disturbances surrounding the breakpoints of a deficiency.

Deletions of chromosomal material occur interstitially and may be considered to disturb the linear order of the genes along the chromosome. Inversions also occur interstitially and likewise result in a disturbance of gene order. A parallel between these two types of aberrations may be drawn. When either is present in heterozygous condition, synaptic difficulties associated with the breakpoints occur. As previously mentioned, difficulty in pairing has been observed cytologically in the case of heterozygous deficiencies. Both cytological and genetic evidence for this have been shown for inversions by Sturtevant and Beadle (1936), who studied X chromosome inversions in relation to their effects on both crossing over and nondisjunction. In inversion heterozygotes, synaptic difficulties are reflected in recombinational disturbances. Recombination outside the inverted segment (areas analogous to chromosomal regions adjoining the breakpoint of a deficiency) is regularly reduced.

The C1B inversion with its left breakpoint
between echinus (ec) and bifid (bi), leaving the region from yellow (y) to ec in normal sequence, was among those tested by Sturtevant and Beadle. A value of 1.29% was reported for the region distal to the inversion by Gershenson (1935). The standard map distance for the y-ec interval is 5.5. Sturtevant and Beadle found that the regions between inversions and the ends of the chromosome exhibited a reduction in recombination, the extent of which depended on whether the region was distal or proximal. Inversions were more effective in suppressing crossing over in distal than in proximal segments. This may reflect differences in the strength or point of initiation of pairing.

Inversion homozygotes (having structural homology between the two chromosomes of the pair) showed about the same total amount of crossing over as homozygous normals. The distribution of crossing over was altered only by the relation of tested intervals to the centromere. Less crossing over occurred near the centromere than at the free end of the chromosome, whether in inversion homozygotes or in normals. These findings point to non-homology between partner chromosomes as a major factor in influencing recombination, but the location of a given region with respect to the centromere is also of significance.
Beadle (1932) originally observed the so-called "centromere effect." In studies on homozygous III-IV translocations, the centromere was found to interfere with crossing over in its immediate vicinity. When gene loci were moved closer to or further from the centromere, their crossing over with neighboring genes was altered accordingly. Graubard (1932), through the use of an inversion, confirmed Beadle's results, making it quite clear that the position of the genes, not the genes themselves, determine the frequency of crossing over.

Other factors besides location of the gene relative to the centromere are known to influence recombination in structurally normal chromosomes. Swanson (1957, 1967) has reviewed these factors. Sex of the individual tested is a well-known factor; suppression of crossing over in *Drosophila* males is an extreme example. Recombination has also been shown to vary markedly with the age of the individual. Crossover frequencies in *Drosophila* females fluctuate characteristically during successive 10-day periods. The first and third such periods of adult life show approximately equal (the first 10 days somewhat higher) recombinational values for normal chromosomes. A marked decrease is found in females 10 to 20 days of age. Variations in temperature also affect recombination, with the greatest effect
evidenced in the region of the centromere. The heterochromatin associated with centric areas also has a possible influence on crossing over in neighboring euchromatic regions. This effect is relatively undefined since its response to the other factors (age, temperature) seems unstable. The so-called "centromere effect" may, in fact, be a product of the large heterochromatic blocks known to occur in association with the centric regions.

Dobzhansky (1934) investigated the effects of duplications on recombination in homologous and non-homologous regions of structurally normal chromosomes. All except one duplication studied were either free or translocated fragments, having one normal telomere. A single insertional duplication was investigated. The results of his studies showed that duplicating fragments reduced recombination in regions homologous to themselves in proportion to their genetic length. Although the insertional duplication showed less influence than the other duplications, this was attributed to its small size. Competition for pairing between the homologous chromosomes and the duplication was considered to be a feasible explanation of the results.

Grell (1964) also reported findings supporting the competitive pairing theory. His results, in addition, demonstrated the importance of the location of a
duplication in affecting recombination. The location of a duplication in relation to its region of homology influenced the probability of effectively competing for pairing: the more removed a duplicating fragment, the less the effect observed. This finding would indicate that insertional duplications are under a severe pairing handicap. The results of both previous studies suggest that short insertional translocations would have little effect on recombination in structurally normal chromosomes.

The effects of the presence of a structurally nonhomologous pair on recombination between other, homologous pairs of the same nucleus were investigated by Steinberg and Fraser (1944). The effects of X chromosome inversions, which stimulated crossing over in the third chromosome, were shown to bear no relation to the size of the inversion or its position relative to the centromere or telomere. The magnitude of an interchromosomal effect was related to the fraction of the total chromatin of the cell that was contained in the affected chromosome. This was viewed as consistent with a physiological theory ascribing interchromosomal effects to a kind of position effect.

A mechanical approach was taken by Schultz and Redfield (1951). Their studies indicated that
concomitant with the presence of an inversion in one pair of chromosomes, a striking increase of crossing over in another pair could be detected. The increase was greatest in regions immediately adjacent to the centromere. Each inversion was shown to possess its own unique specificity of action, but no consistent correlation with size was found. Each nonhomologous pair affected also exhibited a different degree of increase in recombination. These "interchromosomal effects" were thought to reflect alterations of synaptic patterns. In turn, heterochromatin was held to be important in setting the pairing pattern in the nucleus by association of heterochromatic regions.
CHAPTER III

MATERIALS AND METHODS

Crossing over in the X chromosome was measured by using the following markers (described by Bridges and Brehme, 1944): yellow (y) 0.0, white-apricot (w^a^) 1.5, split (spl) 3.0, ruby (rb) 7.5, lozenge (lz) 27.7, and miniature (m) 36.1. These markers were contained in three laboratory stocks: (1) y w^a^ spl rb; (2) lz^50-130^ m (females homozygous for this lz allele, provided by M. M. Green, are fertile); (3) wild type (+) M56i. Deficiencies and balancing segments used are described below.

**Deficiency white-marbled Notch-63b Df(w^ma^N63b):** an x-ray-induced Notch deficiency extending from 3C3 through 3E2, inclusive (18 salivary chromosome bands on Bridges, 1938, revised salivary chromosome map), which is accompanied by a simultaneously elicited, nonuniformly pigmented, white allele, called "marbled," that has properties similar to those of the allele, spotted-white. The deficiency acts as a recessive lethal, expressing a Notch phenotype in the heterozygous condition, and includes the loci of roughest (rst, 1.7), spl
(3.0), and diminutive (dm, 4.6). Heterozygous females are viable and fertile. The deficiency is lethal in males (Lefevre and Wilkins, 1966). In the interest of brevity, hereafter this aberration will be referred to as Df(w^{maN}).

**Deficiency of white through echinus Df(w-ec)^{64d};** an x-ray-induced deficiency extending from 3C2 through 3F1-2 (27 salivary chromosome bands). The deleted segment was simultaneously inserted in section 37D of chromosome 2, Dp(1;2L) (w-ec)^{64d} (Lefevre and Wilkins, 1966; right breakpoint corrected from the published information by Lefevre, personal communication). Males with both the deficiency and the translocated segment inserted in the second chromosome survive and express both a white and echinus phenotype, with normal wings. The left and right break points of this mutant coincide closely with the loci of w(1.5) and ec(5.5), respectively. Females heterozygous for the deficiency express Notch. In referring to this aberration, the abbreviation (w-ec) will be used hereafter, preceded by either Df or Dp to designate the deficient or duplicated condition, respectively.

**Duplication w^{+51b7} Dp(1;2R)w^{+51b7};** a segment extending from 3C2 through 3D5-6, produced by
irradiation of inversion white mottled-4 (\textit{wm^4}), and including some adjacent proximal heterochromatin (but not 3C1). This deleted material was inserted in a euchromatic region of the right arm of chromosome 2 at 54F (Lefevre, 1952). The duplication includes the loci of \textit{w}, \textit{rst}, \textit{spl}, and \textit{dm}.

**Duplication white-Variegated cobbled Dpw\textsubscript{VCO}:** an insertional translocation between the X chromosome and chromosome 3 found by Clausen (Bridges and Brehme, 1944). A segment of the X chromosome from 2C1 through 3C4 was inserted in the heterochromatic region of chromosome 3, which itself has a short inversion across the spindle attachment with breaks at 77D4 and 81A (chromocenter); 3C4 adjoins 81A (analysis by Schultz, cited in Bridges and Brehme, 1944).

All test crosses were carried out following a standard procedure in order to minimize variations in recombination frequencies due to maternal age and temperature. Females heterozygous for the X chromosome to be tested were collected as virgins, aged three days, transferred to fresh food vials, and mass mated for 24 hours with males of the same age carrying recessive markers. Mating vials were discarded the following day,
and females were then individually subcultured daily in fresh vials, without males, for a period of three days (fourth, fifth, and sixth days after hatching) in order to sample eggs from the peak egg-laying period. Emerging progeny were counted and scored for crossovers after the cultures had been incubated for 13 days and again after 17 days.

Each experiment and its control were done concurrently, and where possible control flies were drawn from among the siblings of the experimental flies. All experiments were conducted at 25± 1° C., using 8-dram vials containing standard Drosophila medium.
CHAPTER IV

RESULTS

Recombination in the Vicinity of a Heterozygous Deficiency

Recombination was measured in the genetic interval between y and rb in the presence of two different heterozygous deficiencies included within the marked interval (Table 1). Df(w\textsuperscript{ma}N) reduced recombination in the overall test interval to nearly one-fourth (1.36%/7.28%) that of the normal control value for the same interval obtained from structurally normal chromosomes ($x^2 = 93.3$, $P < .001$, d.f. = 1). In the distal part of the interval (y-deficiency), recombination was reduced to 0.77%; in the region from the deficiency to rb, 1.09% recombination was observed.

Df(w\textsuperscript{ec}), which was also tested, reduced recombination in the total y-rb region to one-fifth (1.52%/7.28%) that of the control value ($x^2 = 55.5$, $P < .001$, d.f. = 1). The measured sub-intervals (y-deficiency; deficiency-rb) showed values of 0.56% (7/1251) and 0.96% (12/1251), respectively.

Although Df(w\textsuperscript{ec}), is 9 bands longer than Df(w\textsuperscript{ma}N), the amount of recombination between y and rb
### Table 1

**Crossing Over in the Vicinity of Deficiencies**

<table>
<thead>
<tr>
<th></th>
<th>$y - w$</th>
<th>$w - spl$</th>
<th>spl - rb</th>
<th>$y - rb$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal chromosomes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$yw^{a}$ spl rb</td>
<td>189 (1.86%)</td>
<td>112 (1.23%)</td>
<td>363 (3.98%)</td>
<td>664 (7.28%)</td>
</tr>
<tr>
<td>$y + + spl + rb$</td>
<td>$x^2 = 18.4$*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=9116)</td>
<td>$P &lt; .001$**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Deficiency heterozygotes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$yw^{a}$ spl rb</td>
<td>19 (0.77%)</td>
<td></td>
<td>27 (1.09%)</td>
<td>46 (1.86%)</td>
</tr>
<tr>
<td>(n=1251)</td>
<td>$x^2 = 0.52$</td>
<td></td>
<td>$x^2 = 0.14$</td>
<td>$x^2 = 0.56$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Deficiency homozygotes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$y Df(w^{anN}) +$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ $Dp(w^{ec})$</td>
<td></td>
<td></td>
<td></td>
<td>70 (3.92%)</td>
</tr>
<tr>
<td>(n=1784)</td>
<td></td>
<td></td>
<td></td>
<td>$x^2 = 0.044$</td>
</tr>
<tr>
<td>$y Df(w^{anN}) +$</td>
<td></td>
<td></td>
<td></td>
<td>35 (4.10%)</td>
</tr>
<tr>
<td>+ $Dp(w^{ec})$</td>
<td></td>
<td></td>
<td></td>
<td>$x^2 = 0.75$</td>
</tr>
<tr>
<td>(n=854)</td>
<td></td>
<td></td>
<td></td>
<td>$P &lt; 0.90$</td>
</tr>
</tbody>
</table>

* $x^2$ and $P$ values lie between entries, the significance of whose differences they test.

** These values are significant at the 95% confidence level.
(1.52% and 1.86%) in the two experiments was not significantly different ($x^2 = .56, .25<P<.50, \text{d.f.} = 1$). Nevertheless, a lower value of recombination was observed with the longer deficiency, as would be expected.

Recombination in the Vicinity of a Homozygous Deficiency

Females possessing $Df(w^{ma}N)$ in both $X$ chromosomes were also tested for recombination in the interval from $y$ to $rb$. Females carrying two deficient chromosomes, one marked with $y$ and the other with $rb$, were provided with a duplicating segment to cover the deleted region, so that they would be viable and fertile. As an examination of Table 1 indicates, a value of 3.92% for the surrounding $y$-$rb$ interval was evidenced in homozygous deficient females when the balancing segment, $Dp(w-ec)$, was present in only one member of the pair of second chromosomes: $y Df(w^{ma}N) +/- + Df(w^{ma}N) rb; Dp(w-ec)/+$. In this cross, it was impossible to determine whether any given $y$-$rb$ recombinant resulted from crossing over in the distal or proximal subinterval. The recombination value represents a significant reduction to almost one-half (3.92%/7.28%) that obtained for the same interval in structurally normal chromosomes.

A similar recombinational value (4.10%) was evidenced in the encompassing $y$-$rb$ interval of $Df(w^{ma}N)$ homozygotes when the balancing segment was present in both
second chromosomes: y Df(w^maN) +/- Df(w^maN) rb; Dp(w-ec)/ Dp(w-ec). The values obtained for recombination in the y-rb interval in the deficiency homozygotes, whether heterozygous or homozygous for the balancing segment, Dp(w-ec), (3.92% vs. 4.10%), did not differ significantly from one another (x^2 = 0.044, 0.50 < P < 0.75, d.f. = 1) and may be pooled for purposes of discussion. When the data from both experiments are combined, a value of 4.03% (105/2638) for the y-rb interval results. This value is significantly greater than the value of 1.86% obtained with Df(w^maN) heterozygotes (x^2 = 20.0, P << 0.001, d.f. = 1).

Recombination in the Presence of Insertional Duplications

Despite their manner of origin, translocated segments of one chromosome inserted into nonhomologous chromosomal regions, when present together with structurally normal chromosomes possessing regions of homology with the segment, may be regarded as duplications. Tables 2 and 3 record the results of the effects of the presence of three different insertional duplications on recombination in normal X chromosomes, both in homologous regions falling within the y-rb interval and in a nonhomologous region (1z-m).

A. Effects on homologous regions: The results of tests of the three duplications on a chromosomal region
Table 2
Crossing Over in a Region Homologous to Insertional Duplications

<table>
<thead>
<tr>
<th></th>
<th>y-w</th>
<th>w-spl</th>
<th>spl-rb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control y w^a spl rb</td>
<td>50 (2.30%)</td>
<td>21 (0.96%)</td>
<td>90 (4.13%)</td>
</tr>
<tr>
<td></td>
<td>x^2 = .96</td>
<td>x^2 = .31</td>
<td>x^2 = .48</td>
</tr>
<tr>
<td></td>
<td>.25&lt;P&lt;.50</td>
<td>.50&lt;P&lt;.75</td>
<td>.25&lt;P&lt;.50</td>
</tr>
<tr>
<td>Dpw^+5lb7 ; y w^a spl rb</td>
<td>23 (1.79%)</td>
<td>10 (0.78%)</td>
<td>56 (4.29%)</td>
</tr>
<tr>
<td></td>
<td>x^2 = .81</td>
<td>x^2 = 1.5</td>
<td>x^2 = .58</td>
</tr>
<tr>
<td></td>
<td>.25&lt;P&lt;.50</td>
<td>.10&lt;P&lt;.25</td>
<td>.25&lt;P&lt;.50</td>
</tr>
<tr>
<td>Control y w^a spl rb</td>
<td>83 (2.22%)</td>
<td>63 (1.66%)</td>
<td>164 (4.39%)</td>
</tr>
<tr>
<td></td>
<td>x^2 = .98</td>
<td>x^2 = 1.5</td>
<td>x^2 = 3.8</td>
</tr>
<tr>
<td></td>
<td>.25&lt;P&lt;.50</td>
<td>.50&lt;P&lt;.75</td>
<td>.25&lt;P&lt;.50</td>
</tr>
<tr>
<td>Dp(w-ec) ; y w^a spl rb</td>
<td>38 (2.65%)</td>
<td>31 (2.16%)</td>
<td>56 (3.91%)</td>
</tr>
<tr>
<td></td>
<td>x^2 = 9.8</td>
<td>x^2 = 3.8</td>
<td>x^2 = 9.8</td>
</tr>
<tr>
<td></td>
<td>P&lt;.001*</td>
<td>.25&lt;P&lt;.50</td>
<td>.10&lt;P&lt;.25</td>
</tr>
<tr>
<td>Control y w^a spl rb</td>
<td>56 (1.75%)</td>
<td>29 (0.90%)</td>
<td>109 (3.40%)</td>
</tr>
<tr>
<td></td>
<td>x^2 = 9.8</td>
<td>x^2 = 7.3</td>
<td>x^2 = 1.3</td>
</tr>
<tr>
<td></td>
<td>P&lt;.001*</td>
<td>.25&lt;P&lt;.50</td>
<td>.10&lt;P&lt;.25</td>
</tr>
<tr>
<td>Dpw^vco ; y w^a spl rb</td>
<td>3 (0.33%)</td>
<td>11 (1.22%)</td>
<td>38 (4.22%)</td>
</tr>
</tbody>
</table>

*This value indicates a statistically significant difference.
Table 3
Crossing Over in a Region of Nonhomology to Insertional Duplications

<table>
<thead>
<tr>
<th></th>
<th>lz +</th>
<th>+ m</th>
<th>Total</th>
<th>Tests of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>289</td>
<td>309</td>
<td>598 (7.94%)</td>
<td></td>
</tr>
<tr>
<td>Dpw +51b7</td>
<td>74</td>
<td>86</td>
<td>160 (6.81%)</td>
<td>x² = 3.0, .05&lt;P&lt;.10</td>
</tr>
<tr>
<td>Dp(w-ec)</td>
<td>41</td>
<td>35</td>
<td>76 (8.77%)</td>
<td>x² = .69, .25&lt;P&lt;.50</td>
</tr>
<tr>
<td>Dpw Vco</td>
<td>103</td>
<td>124</td>
<td>227 (7.13%)</td>
<td>x² = 1.9, .10&lt;P&lt;.25</td>
</tr>
</tbody>
</table>

*Total of the separate controls done simultaneously with the three experiments. Statistical tests showed them to be homogeneous (x² = 3.95, .10<P<.25 d.f. = 2)
homologous to themselves, together with their control values, are listed in Table 2. Since statistical analysis indicated that the control data were not homogeneous and therefore could not be pooled, crossover values obtained in the presence of each duplication were compared only with their respective control values. Neither $D_{p}^{w+51b7}$ nor $D_{p}(w-ec)$ produced any significant effect on recombination values in any of the test intervals ($y-w$; $w-spl$; $spl-rb$) as compared with those found in their respective controls. In contrast, $D_{p}^{vco}$ produced a significant decrease (0.33%/1.75%; $\chi^2 = 9.8$, $P << .001$, d.f. = 1) in the distalmost interval ($y-w$) as compared with the values found in its control. The other intervals tested, $w-spl$ and $spl-rb$, showed no statistically significant linkage disturbances in the presence of $D_{p}^{vco}$.

B. Effects on a nonhomologous region: Table 3 contains recombination values obtained from crosses made to investigate the effects of the same three insertional duplications on a nonhomologous ($1z-m$) interval of the X chromosome. Since the control values in this experiment were shown to be homogeneous ($\chi^2 = 3.95$, .10$<P<.25$, d.f. =2), they were pooled, and the control value given is the average of all three control experiments done simultaneously with their respective test crosses. None of the duplications tested produced any significant effect on
recombination values in the nonhomologous chromosomal region.
Recombination in Heterozygous Deficiencies

Recombination in marked regions of a chromosome encompassing a deficiency in the heterozygous condition should be reduced by at least the genetic length of the missing segment. If, in addition, the deficient chromosome encounters difficulties in pairing with its structurally normal homologue in the regions adjacent to the missing segment, even greater reduction would be anticipated. This latter expectation was realized in the present study.

The amount of recombination observed in the $y$-$rb$ interval in females heterozygous for $Df(w^{ma}N)$ was 1.86%; a value of 7.28% was obtained for the same interval measured in structurally normal chromosomes. (The standard map interval for $y$ to $rb$ is 7.5.) If there were no synaptic difficulties present in the deficiency heterozygotes, then these results could be used to estimate the genetic length of the missing segment. The difference between the two values (5.42%), however, is much greater than the presumed extent of $Df(w^{ma}N)$, judging from its cytological length.
The left breakpoint of \( \text{Df}(\text{w}^{\text{ma}}N) \), as shown by cytological analysis (Lefevre and Wilkins, 1966), lies just to the left of 3C3, leaving 3C2 (and the \( w \) locus it contains) undeleted. As a consequence, the \( y-w \) interval remains completely intact in the deficient chromosome. This fortuitous circumstance provides an opportunity to test recombination in a structurally normal region immediately adjacent to the breakpoint of the deleted segment. Recombination in the \( y-w \) interval in structurally normal chromosomes was shown to be 1.86%; the same interval adjoining \( \text{Df}(\text{w}^{\text{ma}}N) \) exhibited a value of 0.77%. Thus, the \( y-w \) region, when immediately adjacent to the breakpoint of a deficiency, suffered a 59% reduction in recombination.

The second deficiency tested, \( \text{Df}(w-\text{ec}) \), provided additional evidence for reduced recombinational values in the vicinity of deleted segments. Unlike \( \text{Df}(\text{w}^{\text{ma}}N) \), \( \text{Df}(w-\text{ec}) \) includes one band of the \( y-w \) interval, 3C2. This deletion, only one band out of a total of 98 in the \( y-w \) interval, is small enough to ignore in making comparisons with the results obtained with \( \text{Df}(\text{w}^{\text{ma}}N) \). The recombinational values observed with \( \text{Df}(w-\text{ec}) \) for the \( y-w \) region were, in fact, not significantly different from those obtained with \( \text{Df}(\text{w}^{\text{ma}}N) \). Thus, the large, but similar reductions in recombination observed for the \( y-w \)
region with both deficiencies, as compared with controls, indicate that these deficiencies truly inhibit recombination in their vicinities; it is unlikely that the reduced values stemmed from some uncontrolled factor present in both experiments.

Both deficiencies tested remove band 3C7 and, with it, the spl locus found there. Consequently, the spl-rb region tested actually comprised the interval remaining between the right breakpoints of the respective deletions and the ruby locus. Df(w^maN) removes 14 bands from the spl-rb interval; Df(w-ec) lacks 8 more, making a total of 22 bands deleted from the spl-rb interval. A recombinational value of 1.09% for the spl-rb interval was evidenced with Df(w^maN) in heterozygous condition; a value of 0.96% for the same interval was found with Df(w-ec). Although both of these values contrast sharply with the control value of 3.98% found with normal chromosomes, the two values do not differ significantly from one another (x^2 = .14, .50<P<.75), even though one would expect the value with Df(w-ec) to be lower than that observed with the shorter deficiency, Df(w^maN). This result possibly reflects uncontrollable variations in recombination which may occur from experiment to experiment in this type of study. It may, on the other hand, merely signify that an insufficient number of progeny
were counted and classified to establish the significance of the difference.

Bridges (1917), in the initial observation of the deficiency phenomenon, reported the results of genetic tests made in an attempt to localize a segment suspected of being lost from the X chromosome. His results seemed to show a loss of recombination in heterozygous deficient females no greater than the genetic length of the deficiency, although its actual cytological extent was not known. Later, salivary chromosome analysis by Sutton (Bridges and Brehme, 1944) revealed that the deletion Bridges studied had occurred in a chromosome carrying the tandem duplication, Bar. The actual amount of missing material, when compared with a normal (nonduplicated) chromosome, was found to be just 10 salivary chromosome bands (15Fl-9). Bridges credited the deficiency with removing 0.7 of the 4.4 crossover units in the surrounding rudimentary (55.1) to fused (59.5) interval. The short extent of the deficiency and the likelihood of a "centromere effect" (Beadle, 1932) due to the proximity of the test interval to the centromere (66.0) may have contributed to his failure to identify a reduction in recombination in the measured intervals greater than that anticipated from the length of the deficiency.

The Notch-8 ($N^8$) deficiency, lying within the
X-rib interval of the X chromosome and of the same cytological length (18 bands) as Df(w^maN), was studied by Mohr (1923). He reported it to reduce recombination by 3.8% in the surrounding region when present in the heterozygous condition. Because of the state of genetic knowledge at the time the study was carried out, the probability of experimental error was great. Many uncontrolled variables, including some which could have resulted in his failure to identify certain recombinants, were present.

Later, Bridges, Skoog, and Li (1936) reported that a deficiency in the second chromosome having a much greater extent than any previously studied yielded a close correlation between the amount deleted and the reduction in recombination observed. Although the results of genetic tests were somewhat erratic, the 50-band deficiency was estimated to reduce recombination by 1.5% in the area of the second chromosome from which it was deleted. To calculate a theoretical expectation for recombination in the 50 bands, the fraction of the total length of the salivary chromosome map of 2L that was comprised by the deficient region (measured in microns) was multiplied by the genetic length of the whole chromosome arm (in crossover units). Thereby, an expected recombinational value of 1.2% for the missing
segment was obtained. The authors noted the inexactness of the method used to calculate the expected value and admitted the possibility that recombination may have been reduced in the immediate vicinity of the deficiency. Nonetheless, reduction in recombination by more than the genetically measured extent of the deficiency would not be anticipated from the conclusions of Bridges and Mohr. However, unpublished data from later genetic studies indicate that in some cases, at least, a greater reduction does occur (B. H. Judd, personal communication).

Recombination in Homozygous Deficiencies

Homology between chromosomes of a pair provides for their normal synapsis and recombination. Complete homology exists between two X chromosomes when both are lacking the segment deleted by Df(w^maN). The deficient chromosomes should exhibit no loss of recombination as a result of asynapsis in the chromosomal regions that surround the breakpoints of the deleted segments. This expectation was realized when recombination was measured in the surrounding v-rb interval in Df(w^maN) homozygotes. Homozygous Df(w^maN) females must, however, carry a balancing segment, Dp(w-ec), in order to survive and be fertile. As a result, although the X chromosomes are homologous, the second chromosomes may or may not exhibit
homology between one another. The presence of Dp(w-ec)
in only one of the members of the second chromosome pair
results in the establishment of nonhomology between the
two second chromosomes. In this condition, a value of
3.92% for recombination in the y-rb interval was found.
The same interval in structurally normal chromosomes gave
a value of 7.28%.

The existence of a structurally nonhomologous pair
in a nucleus is known to be able to increase recombination
between the remaining homologous chromosome pairs in the
same nucleus. Such an "interchromosomal effect" (Schultz
and Redfield, 1951) could contribute to the recombinational
value observed in the homozygous deficient females
when only one of the second chromosomes contained Dp(w-ec).
If this were the case, the observed value would include,
to some degree, an artificial increment in recombination.

This kind of interaction between nonhomologous
chromosome pairs will not be present when females are
homozygous both for deficient X chromosomes and for
second chromosomes containing the inserted segment. Such
females possess complete structural homology between the
members of the two pairs of chromosomes involved. When
tested, they gave a value of 4.10% for the y-rb interval.
No significant difference in recombination in the test
interval exists between this value and that (3.92%)
observed in the presence of a nonhomologous second chromosome pair ($x^2 = 0.044, \; 0.75 < P < 0.90, \; d.f. = 1$). Because the two values are not significantly different, they may be pooled to give a combined value of 4.03% for the $y$-$rb$ interval. When this figure is compared with that found in the heterozygous condition of $Df(w^{ma}N)$ for the same interval (1.86%), a highly significant difference is evidenced ($x^2 = 20.0, \; P < 0.001, \; d.f. = 1$). The increase in recombination evidenced when structural homology is restored indicates that the value for the $y$-$rb$ interval obtained from the test of homozygous deficient females provides a more accurate estimate of the actual genetic length of $Df(w^{ma}N)$ than do the results of testing heterozygous deficient females. The genetic length of the deleted region, using data from deficiency homozygotes, is 3.25%.

**Cytological and Genetic Correlations**

As genes were localized to specific bands on the salivary chromosome maps, the disproportional spatial distribution of crossovers along the physical length of the chromosome became evident. The lack of correspondence, except for linear order, between genetic and cytological maps necessitates finding a method by which the two units of measurement can be combined to give an accurate picture
of the recombinational nature of the chromosome. Figure
1 provides a plot of the variation in crossing over per
salivary chromosome band for a region of the X chromosome
that includes the test interval used in the present study
(\( \text{y-rb} \)). The values were calculated for intervals de­
limited only by genes whose cytological positions and
standard map locations have been firmly established.
For this reason, the region shown extends beyond the most
proximal marker used in the present study, ruby (whose
exact location is not known), to the nearest accurately
localized gene, rugose. The cytological extents of the
deficiencies and duplications used in these investigations
(including \( \text{N}^8 \) for comparison) are superimposed on the
graph.

The genetic length of a particular deficiency or
duplication can be calculated by multiplying the number of
bands within its extent by the crossover frequency per band
for that particular interval. By this method, a calculated
value of 3.5% is obtained for the 18 bands removed by
\( \text{Df(wmaN)} \). This figure is in close agreement with the
genetic length of \( \text{Df(wmaN)} \) estimated from the reduction
in recombination in the \( \text{y-rb} \) interval obtained with the
deficiency homozygotes (3.25%). By contrast, if data from
\( \text{Df(wmaN)} \) heterozygotes were used, a length of 5.42% would
be predicted. The striking difference between this value
and the calculated value of 3.5% confirms that deficiency heterozygotes exhibit a reduction in recombination by more than the actual genetic length of the missing segment.

Calculations based on the crossover units per band of Df(w-ec) show it to encompass 4.0 crossover units. (Its breakpoints coincide closely with the loci of w and ec at 1.5 and 5.5, respectively, on the standard map.) Unfortunately, difficulty in obtaining sufficient numbers of properly marked Df(w-ec) homozygotes prevented this prediction from being tested.

The highly localized variability in crossover frequencies within the interval used for the present study is well illustrated in Figure 1. This fact dictates that all chromosome regions studied should be similarly evaluated in combined genetic-cytological units before any realistic prediction of the genetic extents of other deficiencies can be made. The extreme distal location of the chromosomal region used for these investigations minimizes any possible centromere effect; however, the influence of the telomere, if any, should be strongly felt. In contrast, more proximal regions, such as those investigated by Bridges (1917, 1936), may be under the influence of the centromere. This effect might result in as great or greater variability in crossover frequencies per band as that observed in the y-rb interval.
Recombination in the Presence of Insertional Duplications

The duplicating segment employed in this study, $Dp(w-ec)$, introduced a variable with which no previous study on the recombinational effects of deficiencies has dealt. As a consequence, further experiments were necessitated to test the effects of $Dp(w-ec)$ and two other insertional duplications on recombination in homologous and nonhomologous regions of structurally normal chromosomes. The results of these experiments insured that the outcome of the present study was not influenced by any interchromosomal effects resulting from the presence of the duplication.

Figure 1 includes the three duplications tested. Multiplying the number of bands found in each duplication by the crossover frequency for their respective regions permits an estimation of the genetic length of each. Using this method, one can conclude that $Dp^{+51b7}$ ought to contain 3.4 crossover units in its 17 bands; $Dp(w-ec)$ 4.0 crossover units in its 27 bands. The physically longest duplication, $Dp^{Vco}$, proved to be the shortest genetically, containing only 1.5 crossover units in 43 bands. The presence of either $Dp^{+51b7}$ or $Dp(w-ec)$, both inserted in euchromatic areas of chromosome 2 (2R and 2L, respectively), produced no significant effect on recombination in homologous regions of structurally normal
chromosomes. However, Dp\textsubscript{wVco}, inserted in the centric heterochromatin of chromosome 3R, elicited a decrease in the distal y-w interval measured. None of the three duplications provoked any effect on a nonhomologous region, l2-m.

The lack of a general increase in recombination in the presence of these insertional duplications excludes the possibility of their generating measurable inter-chromosomal effects. The nonhomology created between the normal homologue and its partner carrying one of the duplications tested must be insufficient to cause such an effect. In the particular case of Dp\textsubscript{wVco}, however, the third chromosome in which it is inserted also possesses a short pericentric inversion. Many inversions are known to produce interchromosomal effects (Schultz and Redfield, 1951). The failure to detect an effect in the presence of this inversion can most likely be traced to the nature of the inversion. The breakpoints occur at 77D4 and 81A (chromocenter), the centromere being inverted. Although the left breakpoint occurs just outside the heterochromatin-euchromatin junction of 3L at the locus of inturned (in, 47.0), the rearrangement primarily involves only heterochromatin. The relationships between the euchromatic elements of the third chromosome remain essentially unaltered by the presence
of the inversion. The lack of an interchromosomal effect may stem from this fact; however, studies on recombination in neighboring regions of the third chromosome would be desirable to verify it.

In studies by Dobzhansky (1934), duplications were shown to decrease recombination in homologous regions in proportion to their length. The majority of the duplications that he studied existed as free or translocated fragments; only one insertional duplication was included in his tests. It proved to have only a slight effect, which he attributed to its small size. All results were viewed as consistent with the theory of competitive pairing. Later, Grell (1964) found that both length and location of a duplication in relation to its region of homology were important in competitive pairing. This provided an additional explanation for the small effect observed in the presence of the one insertional duplication studied by Dobzhansky.

The decreased recombination in the $y$-$w$ region elicited by $D_p^{VCO}$ agrees with these findings. Its physical length is apparently great enough to allow at least some competitive pairing to occur with the homologous region of the normal $X$ chromosome. Because of the smaller physical dimensions of $D_p^{w+51b}$ and $D_p(w-ec)$, their insertional locations, or the combination of both, little
if any pairing occurs with normal X chromosomes in the regions of homology. The inability of all three duplications to affect recombination in the nonhomologous 1z-m interval is also to be expected from the findings of both previous investigators on competitive pairing.
BIBLIOGRAPHY
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